氏　　名	ZHOU XUZHAO
学位の種類	博士（ 医学 ）
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審査研究科	人間総合科学研究科
学位論文題目	Extracellular adenosine and slow-wave sleep are increased after ablation of nucleus accumbens core astrocytes and neurons in mice.（マウスにおける側坐核コア領域のアストロサイトと神経細胞の除去は、細胞外アデノシンと徐波睡眠を増やす。）
主　　査	筑波大学教授　医学博士　高橋　智
副　　査	筑波大学教授　薬学博士　橋本　幸一
副　　査	筑波大学准教授　Ph.D. Neuroscience Vogt Kaspar
副　　査	筑波大学准教授　博士（医学）　坂口昌徳

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Mr. Zhou describes the importance of astrocytes in the nucleus accumbens core for the source of adenosine and sleep regulation. The summary is as follows:

（目的　Purpose）

The role of astrocytes in the nucleus accumbens core in sleep regulation is still unknown. As the first step of this exploration, the author carried out ablation of astrocytes in the nucleus accumbens core and sleep behavior was assessed to better understand the sleep mechanisms controlled by the nucleus accumbens and adenosine.

（対象と方法　Materials and Methods）

The usage of adeno-associated viruses in manipulating genes in special brain regions was well established in mice. By combination of expression of diphtheria toxin receptors using adeno-associated virus and intraperitoneal diphtheria toxin injections (50μg/kg), the author specifically ablated partial astrocytes (also a minority of neurons) in the nucleus accumbens core and checked changes of sleep behavior in both adenosine A_{2A} receptor ($A_{2A}R$) wild-type and knockout mice. Sleep behavior was assessed by the well- established and highly optimized polysomnographic sleep analysis system. The author performed histological studies by immunofluorescent stainings to identify the ablation efficiency and cell status. Moreover, assays of extracellular adenosine levels were conducted by combining *in-vivo* microdialysis in freely moving mice with high performance liquid chromatography analysis.

（結果　Results）

The amount of slow-wave sleep was significantly increased and reached the peak 7 days after ablation happened in the nucleus accumbens core without altering sleep homeostasis in the wild-type mice. Detailed sleep structure

analysis revealed that the increase of slow-wave sleep was due to increased episode numbers in the dark phase but not mean duration. Moreover, the sleep increase disappeared and sleep rhythm returned to a baseline level after 2 weeks. Histological studies revealed that ablation of astrocytes and a minority of neurons in the nucleus accumbens core resulted in highly increased expression of glial fibrillary acidic protein (GFAP), which is a marker for activated astrocytes, and reactivated phagocytic-like microglia. Adenosine assays of dialysates, which were collected by *in-vivo* microdialysis, by using the high performance liquid chromatography system, revealed an increased extracellular adenosine concentration in the ablated mice. However, there was no sleep inducing effect in $A_{2A}R$ knockout mice even if they had ablation and elevated extracellular adenosine levels in the nucleus accumbens.

（考察　Discussion）

The author discusses that as an important metabolite of the cellular energy source molecular- adenosine triphosphate (ATP), adenosine can be produced and released by all kinds of cells. However, where the sleep-associated adenosine originates in the brain is still a mystery. As the research technique develops, a number of proofs start to emerge. Even though the direct proofs to indicate the adenosine source are still lacking, astrocytes are still believed to be a possible source for adenosine in sleep regulation. The author's results discovered an unexpected phenomenon which is that partial ablation of astrocytes (also a minority of neurons) in the nucleus accumbens core can result in astrogliosis, i.e, more active astrocytes. This is considered to be highly relevant with the elevated extracellular adenosine levels which can be the reason of the induced sleep. However, activated microglia was also discovered. The author thinks it is also possible that microglia is a contributor to the increased extracellular adenosine level but there is little evidence due to a lack of reports. Further investigations to demonstrate the ability of astrocytes or microglia in the nucleus accumbens to release adenosine (or ATP) are necessary.

Finally, astrocytic ablation in the central nervous system is unusual in normal physiological conditions whereas in injury or pathological conditions it is quite common. Brain trauma is a representative disease which is usually accompanied by a series of sleep disorders. The author claims that this study may be relevant in clarifying the role of astrocytes and provide a new idea in understanding this syndrome despite further relevant studies and evidences are necessary.

審査の結果の要旨

Abstract of assessment result

（批評　General Comments）

The author used several sophisticated methods, for example adeno-associated viruses in manipulating genes in special brain regions and *in-vivo* microdialysis to analyze the importance of adenosine from astrocytes. Their results suggested the importance of astrocytes in the nucleus accumbens core as the source of adenosine and sleep regulation. These results are important and useful for new treatment development of brain trauma.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on May 24, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.